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Delineating the phenotypic spectrum of Bainbridge-Ropers syndrome: Twelve new patients with *de novo*, heterozygous, loss-of-function mutations in *ASXL3* and review of published literature

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Short Title: Bainbridge-Ropers syndrome: twelve new cases and literature review

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KEYWORDS:

ASXL3, intellectual disability, Marfanoid habitus, heterozygous, loss-of-function

ABSTRACT

Background: Bainbridge–Ropers syndrome (BRPS) is a recently described developmental disorder caused by *de novo* truncating mutations in the Additional sex combs-like 3 (*ASXL3*) gene. To date there have been fewer than ten reported patients.

Objectives: Here we delineate the BRPS phenotype further by describing a series of twelve previously unreported patients identified by the Deciphering Developmental Disorders (DDD) study.

Methods: Trio-based exome sequencing was performed on all twelve patients included in this study which found a *de novo* truncating mutation in *ASXL3*. Detailed phenotypic information and patient images were collected and summarised as part of this study.

Results: By obtaining genotype: phenotype data, we have been able to demonstrate a second mutations cluster region within *ASXL3*. This report expands the phenotype of older patients with BRPS; common emerging features include severe intellectual disability (12/12), poor/ absent speech (12/12), autistic traits (9/12), distinct face (arched eyebrows, prominent

forehead, high-arched palate, hypertelorism and down-slanted palpebral fissures), (9/12), hypotonia (12/12) and significant feeding difficulties (12) when young.

Discussion: Similarities in the patients reported previously in comparison to this cohort included their distinctive cranio-facial features, feeding problems, absent/limited speech, and intellectual disability. Shared behavioural phenotypes include autistic traits, hand-flapping, rocking, aggressive behaviour, and sleep disturbance.

Conclusions: This series expands the phenotypic spectrum of this severe disorder and highlights its surprisingly high frequency. With the advent of advanced genomic screening, we are likely to identify more variants in this gene presenting with a variable phenotype which this study will explore.

INTRODUCTION

Large scale whole exome sequencing projects such as the Deciphering Developmental Disorders (DDD) Project have led to the discovery of a number of new genes underlying developmental disorders [1,2]. A reverse-genetics approach has proven particularly important for the discovery of disorders like Bainbridge–Ropers syndrome (BRPS), whose main clinical features are non-specific, especially when looked at in isolation or with a small number of patients. *De novo* mutation status is the first clue to potential pathogenicity of a given variant and such mutations are known to constitute a significant proportion of the underlying causes of moderate and severe intellectual disability (ID) [3].

ASXL1, *ASXL2* and *ASXL3* are human homologs of the Drosophila additional sex combs (asx) gene that encode putative polycomb proteins and are likely to act as histone

methyltransferases in complexes with other proteins [4]. Polycomb group proteins are implicated in embryogenesis and carcinogenesis through transcriptional regulation of target genes; the *ASXL1* gene is thought to be one of the most frequently mutated genes in malignant myeloid diseases; ASXL is a scaffold protein interacting with methyltransferases and additional proteins of the epigenetic machinery [5,6]. Truncating mutations in *ASXL1* have been reported in association with Bohring-Opitz syndrome (BOS) which has phenotypic overlap with BRPS [7]. More recently, truncating mutations in *ASXL2* were reported in association with a newly recognisable clinical phenotype [8].

Srivastava *et al*., 2016 showed that ASXL3 interacts with BAP1, a hydrolase that removes mono-ubiquitin from histone H2A lysine 119 (H2AK119Ub1) as a component of the Polycomb repressive deubiquitination (PR-DUB) complex [9]. The authors observed a significant increase in H2AK119Ub1 in ASXL3 patient fibroblasts, highlighting an important functional role for ASXL3 in PR-DUB mediated deubiquitination. Transcriptome analysis revealed >500 genes differentially expressed in *ASXL3* patient fibroblasts relative to controls, and these genes were enriched for those involved with molecular processes impacting transcriptional regulation, development and proliferation.

ASXL3 is expressed in similar tissues to *ASXL1* including brain, spinal cord, kidney, liver, and bone marrow, but at a lower level [10]. The high correlation of expression patterns between *ASXL1* and *ASXL3* may account for some of the shared phenotypic features.

Heterozygous, *de novo* loss-of-function mutations in *ASXL3*, underlying the Bainbridge–Ropers syndrome (BRPS: OMIM #615485) have been described in 9 individuals to date [9, 11-13]. The major phenotypic features described in the majority of patients so far include

failure to thrive, global developmental delay, feeding problems, hypotonia, dysmorphic features, profound speech delay and intellectual disability. Here we present genetic and phenotypic information on 12 previously unreported individuals with *de novo* truncating mutations in *ASXL3*, all of which were detected via the trio exome sequencing carried out by the DDD Project. Additional clinical features of BRPS are likely to emerge with identification of additional patients through such large scale exome sequencing projects as described here.

METHODS

EXOME SEQUENCING

In all twelve individuals identified via the DDD study, trio-based exome sequencing was performed on the affected individual and their parents, as previously described by Wright et al., 2014. Each affected individual has also had a high-resolution analysis for copy number abnormalities using array-based comparative genomic hybridization (aCGH). Putative de novo mutations were identified from exome data using DeNovoGear software as described by Ramu et al., 2013 and were validated using targeted Sanger sequencing [14,15].

All recruited patients had the following *de novo* heterozygous pathogenic mutations identified which confirmed the diagnosis of Bainbridge-Ropers syndrome (OMIM:615485):

Patient 1: c.4330C>T, p.(Arg1444*)

Patient 2: c.1201del, p.(Ala401GlnfsTer8)

Patient 3: c.1074T>A, p.(Tyr358*)

Patient 4: c.4144C>T, p.(Gln1382*)

Patient 5: c.1783C>T, p.(Gln595*)

Patient 6: c.3355dup, p.(His1119Profs*7)

Patient 7: c.1082dup, p.(Leu362AlafsTer23)

Patient 8: c.3635T>G, p.(Leu1212*)

Patient 9: c. 3127_3128dup, p.(Gly1045Valfs*99)

Patient 10: c.3178dup, p.(Arg1060Profs*50)

Patient 11: c.1484insTGAA, p.(Asp497*)

Patient 12: c.1491dup, p.(Asn498*)

Mutation nomenclature is according to HGVS recommendations (http://varnomen.hgvs.org/), and is based on reference transcript NM_030632.2.

PATIENT ASCERTAINMENT

All twelve individuals were recruited via UK NHS Regional Genetics Services onto the Deciphering Developmental Disorders (DDD) Project (www.ddduk.org). As part of that study, patient and parental samples receive array CGH and exome sequencing analysis and findings of potential clinical significance are reported back to recruiting clinical geneticists. Any significant findings are usually validated by an accredited UK NHS diagnostic genetics laboratory before being reported to patients and their families; mutations described in this paper have been validated as such. Patient phenotype information was provided to the authors via Clinical Geneticists from several UK NHS genetics services. See Table 1 for a summary of the clinical and molecular findings.

RESULTS

ASXL3 MUTATIONS

Previously reported truncating *ASXL3* mutations cluster mainly within the 5' end of exon 11 between codons 404 and 659. This region lies in-between the N-terminal protein scaffolding functional domains of the gene and the C-terminal chromatin/DNA-targeting functional domain. Srivastava *et al*., 2016 reported two mutations significantly 3' to this main cluster region, at codons 1122 and 1444 [9]. One of the patients (Patient 1) within our cohort carries the same c.4330C>T p.(Arg1444*) mutation as the patient reported by Srivastava *et al*, 2016 suggesting it as a possible recurrent mutation.

Among our cohort, 5/12 (Patients 2, 3, 7, 11, 12) of the mutations could be described as occurring within the originally reported mutation cluster region, 1/12 (Patient 5); c.1783C>T, p.(Gln595*), maps more 3', and the remaining 6 (Patients 1, 4, 6, 8, 9, 10) lie further downstream within the more 3' region, reported by Srivastava *et al*, 2016, which could be considered as a distinct mutation cluster region, extending between codons 1045 and 1444 [9]. All these mutations are publicly available on www.ddduk.org.

PATIENT PHENOTYPES

Antenatal history and birth: Polyhydramnios and concerns regarding poor growth were noted in 1/12 but otherwise unremarkable. For 9/12 patients, a caesarean section was performed, mostly due to breech presentation. All 12 patients had an average birth weight and apart from 4/12 patients who were admitted to the Neonatal unit for respiratory difficulties/ apnoea, the remainder neonatal period was uneventful.

Feeding problems: Consistent with previous reports, 9/12 patients were reported to have significant feeding problems, often including gastro-oesophageal reflux and requiring intervention in the form of nasogastric tube feeding, fundoplication. The majority were described as having failure to gain weight with poor appetite.

Growth: Patients reported here had consistent poor growth with weight and height below the 0.4^{th} centile and relative microcephaly (7/12). This is in keeping with previously reported literature.

Craniofacial features: 9/12 have a high-arched palate, distinctive facial dysmorphism as described below (Figure 1).

Dysmorphic features: 10/12 had down-slanting palpebral fissures and 2 had up-slanting palpebral fissures; both have previously been reported but down-slanting seems to be more common. A long, tubular nose with a prominent nasal bridge is apparent in most. Most of the individuals have a broad nasal tip with low columella. The mouth is wide with full (everted) lower lip. Hypertelorism, a narrow head shape with prominent forehead, 'pencilled' and/or high-arched eyebrows and crowded teeth were also common features.

Other significant features: 12/12 had significant hypotonia, 7/12 had strabismus of varying severity. 3 patients had seizures, previously reported in 2 other *ASXL3* patients. Patient 2 had scoliosis requiring surgery. 3 patients had arachnodactyly, not previously reported in any *ASXL3* patients. 3/12 appears to have a Marfanoid habitus with arachnodactyly, tall stature, pes planus and scoliosis.

Intellectual Disability: The level of intellectual disability ranged from moderate to profound but more likely at the severe end of the spectrum. All patients had ID of varying degree; generally severe. Most were very delayed in walking unassisted and 2 remained entirely non-ambulant. 9/12 patients were entirely non-verbal, including Patient 2 at 22 years of age. 9/12 patients had either formally diagnosed autism or autism spectrum disorder, or were described as having autistic features. 3 patients exhibited hand-flapping, rocking. All the patients were in a special needs school requiring significant help.

Relevant negative findings: Seizures does not appear to be a major feature, seen in only 3/12 patients and generally well-controlled absence seizures; MRI-brain imaging only showed non-specific features with white matter changes (3/12) and vermis hypoplasia (1/12) which is relevant given the significance of intellectual disability in this cohort of patients.

DISCUSSION

The recently described Bainbridge–Ropers syndrome (BRS; OMIM # 615485), associated with *de novo* truncating mutations in the *Additional sex combs-like 3 (ASXL3)* gene (OMIM * 615115), shows phenotypic overlap with Bohring-Opitz syndrome, which is associated with *de novo* truncating mutations in *ASXL1* (OMIM * 612990). Bohring-Opitz syndrome (BOS; OMIM # 605039) is characterised by distinct craniofacial features and posture, severe intellectual disability, feeding problems, small size at birth, and failure to thrive.

Bainbridge *et al*. 2013 reported a series of four unrelated probands with *de novo*, heterozygous, truncating mutations in *ASXL3*, sharing similar phenotypes, including severe feeding difficulties, failure to thrive, and neurologic abnormalities with significant

developmental delay [11]. More recently, truncating mutations in *ASXL2* were reported as being associated with a newly recognisable syndrome with overlapping features to BOS and BRPS [8]. In this report, the authors described six unrelated patients with *de novo* truncating mutations in *ASXL2* with shared clinical features including intellectual disability, macrocephaly, distinct facies, facial nevi, feeding difficulties and hypotonia. Comparison of patients reported in this with BRPS shows the facial dysmorphism to be more similar to BOS with macrocephaly, arched eyebrows, synophrys and facial nevi rather than with BRPS. Other distinguishing features included macrocephaly, congenital heart disease, structural brain malformations and seizures in these patients, which differs to the BRPS cohort. However, there are emerging similarities within this group of conditions, including hypotonia, feeding difficulties and ID, which will become more apparent as more patients are reported with *ASXL2* mutations.

To date there have been fewer than ten reported patients with *de novo* truncating *ASXL3* mutations. Emerging similarities include, distinctive cranio-facial features with arched eyebrows, prominent forehead, high-arched palate, hypertelorism with down-slanted palpebral fissures; significant feeding difficulties needing support; profound/ severe intellectual disability; emerging behavioural phenotype consisting of autistic traits, hand-flapping, rocking, aggressive behaviour, sleep issues with absent/ poor speech. Table 1 provides a comprehensive summary of reported features in this cohort: predominant features in the phenotype are normal pregnancy, higher incidence of caesarean section due to breech presentation, relative microcephaly, significant feeding difficulties, facial dysmorphism , high-arched palate, strabismus, hypotonia, skeletal features including a Marfanoid habitus (especially in the older patients), severe intellectual disability with poor/ absent speech, autistic traits, need for special education. Seizures, structural malformations of internal

organs including the brain, kidneys do not appear to be a predominant part of their phenotype. However, this is likely to be revised/ expanded as more patients are described with BRPS.

There is a wide age range (4-22 years), this being the first report of older patients with BRPS. The older patients in this cohort all have moderate to severe ID, autistic features, attended a special needs school and are in assisted living. Seizures are a component but not a predominant part of their phenotype and they do not appear to have any major structural associations with this diagnosis as they have grown older. The behavioural phenotype appears to be in keeping with other severe developmental disorders with absent/ poor speech, periods of agitation, frustration and poor sleep.

Though Bainbridge-Ropers syndrome (BRPS) is likely to remain a challenging syndrome to recognise clinically, however this cohort of patients has enabled further delineation and expansion of the phenotype. Results of analysis of the first several thousand patient trios within the DDD Project suggests that *de novo ASXL3* mutations are among the more common underlying causes of disease within the DDD cohort (at time of writing, *ASXL3* ranks number 12 out of the top 20 genes in which a pathogenic *de novo* mutation has been found), and therefore it is expected that there will be many more BRPS patients diagnosed in the near future, further defining the associated clinical spectrum.

Our cohort has also firmly established a second, 3' mutational cluster region within *ASXL3* which may be of significance to disease mechanism. In regards to this, an *ASXL3* mRNA transcript carrying the c.1448dupT truncating mutation has previously been shown to be prone to nonsense-mediated decay, with resultant reduction in expression of ASXL3 [6].

Consistent with previous reports and consistent with this disease mechanism, the cohort of patients described here do not show a correlation between phenotypic features or severity and mutation position. It has previously been noted that several truncating mutations in *ASXL3* are described in databases composed of sequence variants from phenotypically normal individuals (see Figure 2). To date there are 4 such mutations within the ExAC dataset, each identified within only one individual within the dataset, and they occur both 5' to the original 5' mutational cluster region (MCR) and 3' to the new 3' cluster region, and also in between the two cluster regions. The explanation for these mutations is as yet uncertain.

We have also been able to collect phenotypic data from several patients with previously unreported missense variants in *ASXL3* (including p.Ser86Ala, p.Lys1026Asn, p.Arg933Trp and p.Ser720Cys). However, in each case the variants were inherited from clinically unaffected parents and the patients had very dissimilar presentations in comparison to the clinical presentation associated with *ASXL3* loss-of-function mutations. Without further investigation it cannot be ruled out that these variants are of clinical significance, however it is unlikely that they are the sole cause of the phenotypes observed in these patients and it is possible that they represent rare polymorphisms. In support of this, all of these variants are found, albeit at low frequency, within healthy control populations (Exome Aggregation Consortium, Cambridge, MA, URL: http://exac.broadinstitute.org).

Ropers *et al*., 2015 previously highlighted the presence of truncating mutations in *ASXL3* and several other dominant genes for intellectual disability or related disorders, within healthy control populations, suggesting the possibility of incomplete penetrance for truncating

mutations within these genes [14]. This included *ASXL1*, for which 56 such mutations were found within the ExAC dataset.

Whilst it cannot be entirely ruled out that truncating *ASXL3* mutations exhibit incomplete penetrance, the number of such mutations (four) found within the ExAC data is still relatively small compared to *ASXL1* and the other genes examined, and the list of reported patients with *ASXL3* truncating mutations that have a Bainbridge-Ropers syndrome consistent phenotype is growing.

Therefore, it seems reasonable that all four of these *ASXL3* mutations may be accounted for by the various other explanations that Ropers put forward, for example, two of the mutations occur at the extreme 3' end of the gene, and may therefore escape nonsense mediated decay (NMD), retaining protein activity. Bainbridge *et al*., 2013 suggested that mutations may arise post-zygotically or during later embryogenesis and thus the phenotypic variability or incomplete penetrance may be explained by mosaicism [11]. With the increasingly wider access of high read-depth exome sequencing for genetic diagnosis of children with developmental disorders it seems likely that this question will eventually be answered as more data emerges.

CONCLUSIONS

In this series, we report 12 patients with *ASXL3* loss-of-function *de novo* variant and expand the phenotype of Bainbridge-Ropers syndrome. New specific associated clinical features have become apparent, such as hypotonia, Marfanoid habitus, and arachnodactyly. This

cohort is consistent with previously reported patients with regards to the facial features and also confirms pertinent negative features such as lack of significant findings on brain imaging, and lack of seizures. This research further reiterates the power of whole exome studies in conjunction with a detailed clinical phenotype in providing an explanation for our patient's difficulties and a unifying diagnosis for their concerns.

STATEMENTS:

A. Funding:

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B. Acknowledgements:

We would like to thank all these families for consenting to publication.

C. Contributorship Statement:

All authors recruited their respective patients to the DDD study and provided data regarding their patients; DDD study provided trio exome sequencing data. MB and JB planned the study; recruited Patient 1 to DDD; wrote manuscript; all authors reviewed and contributed to the manuscript.

D. Competing Interest: None to declare for all authors.

FIGURE AND TABLE LEGENDS

Figure 1: Facies of individuals with *ASXL3* loss-of-function mutations reported herein demonstrating down-slanted palpebral fissures, a long, tubular nose with a prominent nasal bridge is apparent in most. Most individuals have a broad nasal tip with low columella. The mouth is wide with full (everted) lower lip; hypertelorism, a narrow head shape with prominent forehead, 'pencilled' and/or high-arched eyebrows.

Figure 2. Map of ASXL3 mutations reported to date. Mutation nomenclature according to HGVS guidelines (http://varnomen.hgvs.org/) using NCBI reference **Transcript** NM_030632.3).

Table 1: Clinical features of twelve previously-unreported patients with *ASXL3* loss-of-function mutations reported herein in comparison to previously reported patients with Bainbridge-Ropers syndrome.

Supplementary section containing a detailed clinical summary of all patients reported here.

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	Bainbridge *et al*., (2013)* Patient 1	**Bainbridge *et al*., (2013)* Patient 2**	**Bainbridge *et al*., (2013)* Patient 3**	**Bainbridge *et al*., (2013)* Patient 4**	**Dinwiddie *et al*., (2013)**	**Srivastava *et al*., (2016) Patient 1**	**Srivastava *et al*., (2016) Patient 2**	**Srivastava *et al*., (2016) Patient 3**	**Hori et al., 2016**	**Patient 1**
Decipher ID	NA	NA	NA	NA	NA	NA	NA	NA	NA	274629
***ASXL3* Variant**	c.1210C>T, p.Gln404*	c.1396C>T, p.Gln466*	c.1422dup, p.Glu475*	c.1978_1981del, p.Asp660Asnfs*16	c.1897_1898delCA, p.Gln633Valfs*1	c.4330C>T, p.(Arg1444*)	c.3664C>T, p.Q1122X	c.1448dupT, p.Thr484Asnfs*5	c.3039+1G>A	c.4330C>T p.(Arg1444*)
Age	NR	3yrs	5yrs	3.5yrs	6yrs	5.5yrs	4yrs	13m	5yrs	21yrs
Sex	NR	M	F	F	F	F	M	M	F	M
Height	-4.5 to -5SD	<1%	Normal	Reduced	10^{th}- 25^{th}	24^{th}	NR	NR	-3 SD(<<0.4^{th})	50th
Weight	-4.5 to -5SD	<1%	Normal	Reduced	10^{th} – 25^{th}	24^{th}	NR	NR	-3.4SD (<0.4^{th})	75^{th}-91^{st}
OFC	-4.5 to -5SD	<1%	Normal	Reduced	3^{rd}	4^{th}	NR	NR	-3 SD (<<0.4^{th})	25^{th}
Pregnancy	IUGR	Normal	Normal	Normal	Normal	IUGR	Normal	Normal	Normal	Normal
Birth	Normal	Breech	CS	Normal	CS; Breech; BW-1.88kg	Normal; BW- 2.25kg	Normal; BW-3.4kg	Normal; BW- 3.1kg	Normal; BW-2.43kg	Elective CS ; breech; BW-3.7kg
Neonatal Unit	NR	NR	NR	NR	Yes	No	No	No	Yes, 2/52	Yes, 5/7
Gestation	Term	Term	Term	Term	34 weeks	Term	Term	Term	Term	Term
Feeding Difficulties	Yes	Yes	No	Yes	Yes	Yes	Yes	Yes	Yes	No
Cranio-facial features	Arched, thin eyebrows, microcephaly	Arched, thin eyebrows, microcephaly	Frontal bossing, short nose	Arched eyebrows, hypoplastic ale nasi	Microcephaly, trigonocephaly, epicanthic folds	Low-set ears, short nose, micrognathia	Anteverted nares, small chin	NAD	Prominent forehead, thick eyebrows	Long tubular nose, thin upper lip
Downslanting palpebral fissure	NR	Yes	Yes	Yes	No	Yes	Yes	Yes	Yes	Yes
Palate	NR	NR	NR	Narrow, high-arched	Normal	NR	NR	NR	NR	High-arched
Strabismus	NR	NR			No	No	No	No	Yes	No
Hypotonia	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No	Yes
Marfanoid habitus	NA	NR	NR	NR	No	No	No	No	No	Yes
Skeletal features	Ulnar deviation	Ulnar deviation	Normal	Ulnar deviation	Metopic synostosis	NR	Ulnar deviation	No	camptodactyly	arachnodactyly
ID	Severe, died at 9/12	Severe	Moderate	Severe	Severe	Moderate	Moderate	Moderate	Moderate	Moderate
Seizures	NR	NR	NR	NR	No	Yes	No	Yes	No	No
Autism/ ASD	NR	NR	NR	NR	Yes	NR	NR	NR	Yes	Yes (and Tourette's syndrome)
Structural anomalies	NR	NR	NR	White matter changes on MRI	Pulmonary stenosis	White matter changes on MRI	Vermis hypoplasia	White matter changes on MRI	No	No
Dev. Outcome	Delayed	Delayed	Delayed	Delayed	Delayed	Delayed	Delayed	Delayed	Delayed	Delayed
Speech and language	NA	Absent	Absent	Absent	Absent	Absent	Few words	Absent	Delayed, few words	Delayed, needed speech therapy
Behaviour	NA	NR	NR	NR	Poor sleep; aggressive	NR	Aggressive	NR	Moderate autism, poor sleep	Tourette's syndrome, autistic traits
Hand flapping	NA	NR	NR	NR	NR	NR	NR	NR	NR	Yes
Educational support	NR	NR	NR	NR	NR	NR	NR	NR	NR	Yes: SEN

	Patient 2	Patient 3	Patient 4	Patient 5	Patient 6	Patient 7	Patient 8	Patient 9	Patient 10	Patient 11	Patient 12	R/V
Decipher ID	275029	274593	278695	261513	265854	271912	275860	265908	259240	272591	208772	
***ASXL3* Variant**	c.1201del p.(Ala401fs)	c.1074T>A p.(Tyr358*)	c.4144C>T p.(Gln1382*)	c.1783C>T p.(Gln595*)	c.3355dup p.(His1119fs)	c.1082dup p.(leu362fs)	c.3635T>G p.(Leu1212*)	c.3127_3128dup p.(Gly1045fs)	c.3178dupC, p.(Arg1060fs)	c.1484insTGAA, p.(Asp497Ter)	c.1491dup p.(Asn498*)	
Age	22yrs	6yrs	6yrs	9yrs	6yrs	10yrs	20yrs	4yrs 3mths	9yrs	19yrs	8yrs	
Sex	F	F	F	M	M	Male	Female	Female	Male	Female	Male	
Height	<0.4th	25th	9th	25th	75th-91st	50th	2nd	50th	25th	9th	50th	
Weight	0.4th	50th	<0.4th	25th-50th	50th	>99th	<0.4th	0.4th	9th	2nd	25th	
OFC	<0.4th	<0.4th	0.4th-2nd	0.4th-2nd	0.4th	<25th	<0.4th	0.4th	<0.4th	0.4th	50th	
Pregnancy	Polyhydramnios	Normal	Normal	Normal	Normal	Normal	IVF (twin well)	Normal	Mild renal pelvis dilatation at 20/40, resolved postnatally	Normal	Normal	11/12
Birth	Em CS; breech; BW- 2.98kg	Em CS; BW- 3.12kg	Normal; BW- 2.7kg	Em CS; breech; BW- 3.5 kg	Induced	Normal	Induced, Em CS	El CS; breech	El CS; extended breech; BW- 3.4kg	Normal; BW- 3.4 kg	El CS; breech	9/12 CS
Neonatal Unit	Yes, 5/7	No	No	No	Yes, apnoea	No	No	No	No	Febrile	No	4/12
Gestation	Term	Term	Term	39/40	42/40	41 weeks	40 weeks	38/40	38/40	40 weeks	38 weeks	
Feeding Difficulties	Yes: Poor suck/swallow, GOR, fundoplication at 3yrs	No	Yes: Hx of GER, feeding issues, poor appetite	Yes: Stopped breast feeding at 4mths due to poor weight gain	Yes: gastrostomy fed; GOR; fundoplication	No	Yes: Severe, NG fed for 2 yrs	Yes	Yes: GOR; FTT	Yes: frequent vomiting as a baby	Yes	9/12
Cranio-facial features	hypertelorism, hirsutism, finely 'pencilled' eyebrows	hypertelorism, down-slanting palpebral fissures	thick eyebrows, prominent forehead, high nasal bridge, deep set eyes	posteriorly rotated ears, dental overcrowding, long jaw	prominent nasal bridge, tongue tie, micrognathia	synophrys, finely 'pencilled' eyebrows, micrognathia, prominent central incisors	scaphocephaly, prominent nasal bridge, mild synophyrus, crowded teeth, high palate	–	long face with slightly upslanted palpebral fissures and twin hair crowns	Extremely slim and long limbed	–	9/12
Downslant pf	No	Yes	Yes	No	No	Yes	No	No	No	Yes	Yes	6/12
Palate	High-arched	Narrow, high-arched	NAD	High-arched	High-arched	NAD	High-arched, bifid uvula	Normal	High-arched	High-arched, narrow palate	High-arched	9/12
Strabismus	Yes, divergent	Yes	Yes	No	Yes	No	Bilateral, convergent/divergent	Yes	No	No	Yes	7/12
Hypotonia	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	12/12
Marfanoid habitus	No	No	Yes	No	No	No	No	No	_	Yes	No	3/12
Skeletal features	Camptodactyly 4th fingers, short distal phalanx of thumbs, scoliosis	hyperflexible elbows	arachnodactyly	No	No	No	narrow small feet, pes planus	No	postural scoliosis	Malar hypoplasia, long slim hands and feet	No	7/12
ID	Profound	Moderate	Severe	Severe	Severe	Moderate/Severe	Severe	Moderate	_	Severe	Severe	12/12
Seizures	Yes; absences	No	No	No	No	No	Possible absences 6y; GTCS from 11y	No	No	GTCS in childhood	Yes	3/12
Autism/ ASD	Likely; not formal	No	ASD	Likely (no formal Dx)	No	Autism	Autism (Dx 6yrs)	No	ASD, SDP, ADD	Likely (no formal Dx)	Yes	9/12
Structural anomalies	unilateral choanal stenosis	No	No	No	No	No	Bifid nail, laryngomalacia, intestinal malrotation	No	No	No	No	2/12
Speech and language	only noises	v. delayed	v delayed	makes noises and uses Makaton	Communicates by looking at objects	Non-verbal, displays comprehension	Only simple sounds, no words: uses PECS	delayed	Uses PECS	–	no expressive language	12/12
Behaviour	Autistic traits, hyperventilates	No	autistic traits	No	Periodic agitation with self-harming	Poor sleep; difficult behaviour	Frustrated; hand flapping when upset	–	Pica; sleep disorder	Grunting; bruxism	generally placid	9/12
Hand flapping	Yes	No	No	No	No	No	Yes	shakes and claps hands	head rocking and clicking	–	Yes	3/12
Educational support	Yes: special school	Yes: special school	Yes: special school	Yes: special school	Yes: special school	Yes: special school	Yes: special school	Yes: special school	Yes: SEN	Yes: SEN	Yes: special school	12/12

M: Male; F: Female; NR: Not reported; NA: Not applicable; PD: Palpebral fissures; ASD: Autism spectrum disorder; ID: Intellectual disability; Dx: Diagnosis; NAD: No abnormality detected; BW: Birth weight; Em CS: Emergency caesarean section; El CS: Elective caesarean section; SEN: Special educational needs; GOR: gastro-oesophageal reflux; FTT: Failure to thrive; GTCS: generalised tonic-clonic seizures; PECS: Picture exchange communication system; -: Not known; R/V: review summary